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Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in the South-East Queensland region

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***Objectives*: The aim of this study was to determine the antimicrobial resistance patterns of 125 *Campylobacter jejuni* and 27 *Campylobacter coli* isolates from 39 Queensland broiler farms.**

***Methods*: Two methods, a disc diffusion assay and an agar-based MIC assay, were used. The disc diffusion was performed and interpreted as previously described (Huysmans MB, Turnidge JD. Disc susceptibility testing for thermophilic campylobacters. *Pathology* 1997; 29: 209–16), whereas the MIC assay was performed according to CLSI (formerly NCCLS) methods and interpreted using DANMAP criteria.**

***Results*: In both assays, no *C. jejuni* or *C. coli* isolates were resistant to ciprofloxacin or chloramphenicol, no *C. coli* were resistant to nalidixic acid, and no *C. jejuni* were resistant to erythromycin. In the MIC assay, no *C. jejuni* isolate was resistant to nalidixic acid, whereas three isolates (2.4%) were resistant in the disc assay. The highest levels of resistance of the *C. jejuni* isolates were recorded for tetracycline (19.2% by MIC and 18.4% by disc) and ampicillin (19.2% by MIC and 17.6% by disc). The *C. coli* isolates gave very similar results (tetracycline resistance 14.8% by both MIC and disc; ampicillin resistance 7.4% by MIC and 14.8% by disc).**

***Conclusions*: This work has shown that the majority of *C. jejuni* and *C. coli* isolates were susceptible to the six antibiotics tested by both disc diffusion and MIC methods. Disc diffusion represents a suitable alternative methodology to agar-based MIC methods for poultry *Campylobacter* isolates.**

Keywords: MICs, disc diffusion, multiresistance

Introduction

Campylobacter is the most common bacterial cause of foodborne disease in Australia.[1] Contaminated animal products, particularly undercooked or raw poultry meat and raw milk, are recognized as being the primary vehicles of human infections.[2] Although most cases of *Campylobacter* infection are acute and self-limited in nature and do not require antibiotic treatment,[3] antibiotic treatment may be necessary in severe cases or immunocompromised patients.[4]

There is little Australian data on the levels of antibiotic resistance in animal isolates of *Campylobacter*. The only extensive prior Australian study[5] used a disc diffusion method to examine 213 poultry isolates.

We report on the antimicrobial susceptibility patterns present in 125 *Campylobacter jejuni* and 27 *Campylobacter coli* isolates collected from 39 broiler farms in South-East Queensland.

Materials and methods

Bacteria and growth conditions

The 125 *C. jejuni* and 27 *C. coli* isolates used in this study were all confirmed by PCR[6] and were obtained during a large epidemiological study. The *C. jejuni* isolates came from 39 broiler farms, and the *C. coli* isolates came from 14 farms (all farms being a subset of the 39 farms yielding the *C. jejuni* isolates). All the isolates had been genotyped by the *fla*A restriction fragment length polymorphism method.[7] Multiple isolates from a farm were included, provided that the isolates showed different genotypes.

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used as control strains.

Incubation of *Campylobacter* species was performed at 37ºC in a modified atmosphere incubator with a microaerobic

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atmosphere of 5% O_2, 10% CO_2 and 85% N_2. The other bacteria were incubated at 37°C in air.

Antimicrobial susceptibility testing—disc diffusion

The disc diffusion methodology was based on the National Committee for Clinical Laboratory Standards.[8] The disc content was as follows: ampicillin, 10 μg; chloramphenicol, 30 μg; ciprofloxacin, 5 μg; erythromycin, 15 μg; nalidixic acid, 30 μg and tetracycline, 30 μg. All discs were sourced from Oxoid. The isolates were grown on brain heart infusion agar (Becton Dickinson no. 4311037) containing 5% sheep blood cells (BioMerieux no. 04378) at 37°C for 48 h in the modified atmosphere incubator described earlier. The CLSI (formerly NCCLS) method[8] was followed using a growth method inoculum, with the exception that the turbidity of the inoculum was adjusted to the equivalent of a 1.0 McFarland turbidity standard. A purity check, performed by inoculation onto sheep blood agar, was performed for all suspensions. The inoculated Mueller–Hinton agar (MHA) with lysed horse blood (Oxoid no. PP2097) and purity check plates were incubated for 44–48 h at 37°C in the modified atmosphere incubator. For each test run, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were used as control strains. The control strains were tested using MHA (Oxoid no. PP2096) plates incubated aerobically at 37°C. The results for the control strains were read after 18–24 h of incubation. The interpretation of susceptible, intermediate and resistant was based on the criteria of Huysmans and Turnidge[9] (Table 1).

Antimicrobial susceptibility testing—MIC testing

The MIC testing was done by a standardized agar dilution method,[8] with the exception that the turbidity of the inoculum was adjusted to the equivalent of a 1.0 McFarland turbidity standard. The inoculated MHA and purity check plates were incubated for 44–48 h at 37°C in the modified atmosphere incubator. For each test run, control strains (all three listed above) were used (as described above). The results for the control strains were read after 18–24 h of incubation, whereas the *Campylobacter* results were read after incubation for 44–48 h.

Antibiotics were tested in a 2-fold concentration series: ampicillin, 0.25–64 mg/L; chloramphenicol, ciprofloxacin and erythromycin, 0.25–32 mg/L; nalidixic acid, 1–128 mg/L and tetracycline, 0.25–128 mg/L. The presence of growth was determined by visual examination and the MIC defined as the lowest concentration of the antibiotic to prevent growth. Interpretation of the results of the *Campylobacter* isolates was performed using the resistance breakpoints published by DANMAP2004.[10] As DANMAP2004[10] does not contain a breakpoint for ampicillin, we used the resistance breakpoint used by the CLSI for ampicillin resistance in Enterobacteriaceae (≥32 mg/L). The breakpoints are shown in Table 1.

Statistical analysis

The overall level of resistance to at least one antibiotic in the *C. jejuni* and *C. coli* isolates was compared by χ^2 analysis (Statistix Software).

Results

The results of the disc diffusion and MIC testing are shown in Table 2. At all times, the results from the control strains were within the range indicated as acceptable by the relevant CLSI guidelines.[8]

There was a strong agreement in the MIC and disc diffusion methods for all six antibiotics tested for both *Campylobacter* species (Table 2).

The level of resistance to any antibiotic examined in this study never exceeded 20% for either *C. jejuni* or *C. coli*. Among the 125 *C. jejuni* isolates, the highest level of resistance was to tetracycline (19.2% by MIC and 18.4% by disc) and ampicillin (19.2% by MIC and 17.6% by disc). A similar level of resistance to these same two antibiotics was found in the 27 *C. coli* isolates tested (Table 2). A low level of resistance to nalidixic acid (2.4%) was found in the *C. jejuni* isolates by disc, whereas all the *C. jejuni* isolates were susceptible by MIC. All *C. coli* isolates were susceptible to this antibiotic by MIC and disc methods. A low level of resistance (11.1% by MIC and disc) was found to erythromycin among the *C. coli* isolates, whereas

Table 1. Interpretation criteria used in this study

Antibiotic[a]	Zone diameter (mm) indicating[b]			Resistant breakpoint (mg/L)
	S	I	R	
Ampicillin (10 μg)	≥10	n/a	≤9	≥32[c]
Chloramphenicol (30 μg)	≥23	12–22	≤11	≥32
Ciprofloxacin (5 μg)	≥24	19–23	≤18	≥4
Erythromycin (15 μg)	≥19	16–18	≤ 15	≥32
Nalidixic acid (30 μg)	≥15	n/a	≤14	≥32
Tetracycline (30 μg)	≥33	16–32	≤15	≥16

The disc diffusion criteria were suggested by Huysmans and Turnidge,[9] whereas the MIC breakpoints, except where indicated, are from DANMAP 2004.[10]
[a]The figure in brackets is the amount of antibiotic in the disc.
[b]S, susceptible; I, intermediate; R, resistant.
[c]No breakpoint provided by DANMAP 2004.[10] This is the breakpoint provided by NCCLS[8] for Enterobacteriaceae.

Table 2. Results of MIC and disc diffusion tests for 125 *C. jejuni* and 27 *C. coli* isolates

		Distribution (%) of MIC (mg/L)[a]													Distribution (%) of disc results		
Antibiotic	Species	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	% R	S	I	R
Ampicillin	*jejuni*		0	0	4.0	10.4	28.0	35.2	3.2	4.8	11.2	3.2		19.2	82.4	0	17.6
	coli		0	0	11.1	7.4	11.1	22.2	40.7	7.4	0			7.4	85.2	0	14.8
Chloramphenicol	*jejuni*		0	0	0	44.0	31.2	23.2	1.6	0				0	98.4	1.6	0
	coli		0	0	0	33.3	48.1	18.5	0	0				0	100	0	0
Ciprofloxacin	*jejuni*		72.0	24.8	3.2	0	0	0	0	0				0	100	0	0
	coli		66.7	11.1	22.2	0	0	0	0	0				0	100	0	0
Erythromycin	*jejuni*		0	0.8	14.4	50.4	20.0	11.2	3.2	0				0	100	0	0
	coli		0	18.5	14.8	22.2	11.1	18.5	3.7	11.1				11.1	88.9	0	11.1
Nalidixic Acid	*jejuni*				0	16.0	55.2	25.6	3.2	0	0	0		0	97.6	0	2.4
	coli				0	11.1	55.6	33.3	0	0	0	0		0	100	0	0
Tetracycline	*jejuni*		57.6	16.0	6.4	0.8	0	0	1.6	4.0	4.0	2.4	7.2	19.2	80.0	1.6	18.4
	coli		66.7	3.7	14.8	0	0	0	0	0	0	14.8		14.8	85.2	0	14.8

[a]Vertical lines indicate breakpoints for resistance. The white fields denote dilution range tested for each antibiotic. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration.

all *C. jejuni* isolates were susceptible to this agent by both MIC and disc methods.

Resistance to more than one antibiotic was detected by disc diffusion in 9 *C. jejuni* isolates (7.2%) and by MIC in 11 *C. jejuni* isolates (8.8%). All of these isolates were resistant to both tetracycline and ampicillin. By disc diffusion and MIC methods, none of the 27 *C. coli* isolates showed resistance to more than one antibiotic.

The overall level of resistance (by both disc diffusion and MIC methods) was not significantly different in *C. jejuni* and *C. coli*.

There were four broiler farms that contributed nine or more isolates of *C. jejuni* to this study. In all of these cases, the genotyping indicated that—within the farm—all the isolates tested were distinct and different genotypes. The occurrence of resistance to ampicillin and tetracycline was not uniform within a farm—isolates ranging from 11.1% to 30% for ampicillin and from 18.2% to 30% for tetracycline.

Discussion

The on-going studies on the epidemiology of *Campylobacter* in broilers in our laboratory allowed the selection of isolates across a large number of broiler farms (39 in total). At the time these studies were performed, the number of broiler farms in the South-East Queensland region was estimated to be 120. Hence, our study—based on 33% of the existing farms—provides a sound insight into the prevalence of antimicrobial resistance in *Campylobacter* associated with Queensland poultry. Our selection of isolates was further guided by our knowledge, arising from the epidemiological studies, of the different genotypes of *C. jejuni/coli* present within a flock. This knowledge of genotype allowed us to include multiple isolates from within a flock—with the knowledge that each isolate represented a different genotype. In contrast, the prior Australian study[5] was based on isolates obtained from either carcass rinses or intestinal samples—with no information on the genetic diversity or farm of origin available.

The level of resistance we found to tetracycline for *C. jejuni* and *C. coli* was at the lower range of that reported in the prior Australian study (15–36%).[5] Higher levels of tetracycline resistance have been reported from four European Union countries (35.4%)[11] and the USA (43%).[12] In Sweden, where tetracycline has not been added to chicken feed since 1986,[13] the level of tetracycline-resistant *Campylobacter* has been reported to be 1%.[13]

The level of resistance to ampicillin among both our *C. jejuni* and *C. coli* isolates was similar to that reported in other countries such as Germany (20% for *C. jejuni* and 23.5% for *C. coli*)[14] and Canada (22% for *C. jejuni* and 12% for *C. coli*).[15] The prior Australian study reported a much higher level of ampicillin resistance (50.4–63.6%).[5]

The major difference between this study and the majority of similar studies performed in other countries is the absence of resistance to ciprofloxacin. As fluoroquinolones have not been registered for use in chickens in Australia, it was not surprising to find that none of the 125 *C. jejuni* and 27 *C. coli* isolates was resistant to this antibiotic. The prior Australian study reported a similar absence of ciprofloxacin resistance.[5] In contrast, ciprofloxacin resistance has been reported in the USA (19%)[12] and a range of European nations (14.9% of *C. jejuni* and 39.6% of *C. coli* isolates).[11] An absence or near absence of ciprofloxacin resistance has also been reported from Brazil,[16] Canada[15] and Norway.[17]

In contrast to our findings, other studies have reported that *C. coli* isolates show higher levels of resistance than *C. jejuni* isolates.[11,18] In a Northern Ireland study that found no significant

difference between the resistance patterns between *C. jejuni* and *C. coli*, the authors suggested that the uncommon occurrence of pig husbandry on poultry farms in Northern Ireland might explain the lower rate of resistance in *C. coli* isolates.[19] It is notable that none of the broiler farms represented in our study involved co-location with pig husbandry operations.

Our finding of no multiple resistance (defined as resistance to four or more different classes of antibiotics) has been also reported in a number of countries—four European Union countries,[11] Northern Ireland[19] and Sweden.[13]

Our examination of multiple isolates (all genotypically different) within four farms demonstrated that isolates within each farm could be both susceptible and resistant to ampicillin and tetracycline, a finding that has been reported previously for ciprofloxacin.[20]

Overall, we found a good correlation between the disc diffusion methodology of Huysmans and Turnidge[9] and the MIC methodology. For those laboratories that lack the capacity to undertake MIC-based methodologies, disc diffusion represents, in our view, an acceptable method for the determination of antimicrobial resistance patterns in *Campylobacter*.

Our study has provided solid evidence that the majority of Queensland poultry isolates of *Campylobacter* shows little resistance to antibiotics that are either used in the poultry industry or of public health significance.

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Transparency declarations

J. M. T. currently leads a project funded by the Rural Industries Research and Development Corporation (Chicken Meat Program), with some of the funds employing a staff member of the laboratory. P. J. B. is a member of the Chicken Meat Research and Development Committee within the Rural Industries Research and Development Corporation.

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